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Mutagenic Properties of Drinking Water Disinfectants and By-Products

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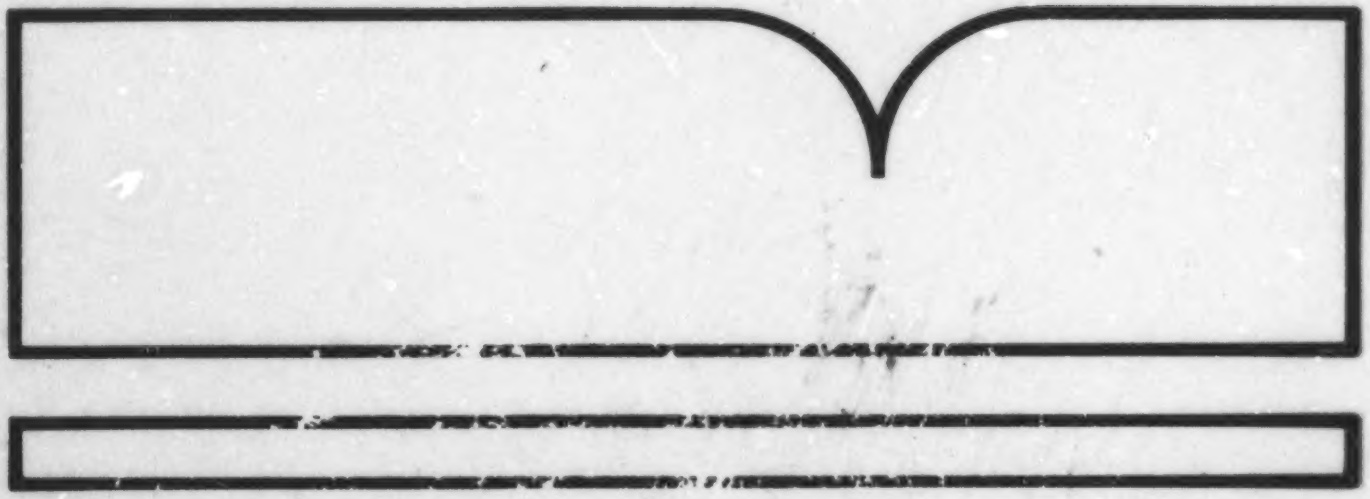

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Mutagenic Properties of Drinking Water Disinfectants and By-Products

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INTRODUCTION

The identification of a number of mutagenic and carcinogenic chemicals in our public water supplies has raised concern over potential genetic and carcinogenic hazards to the human population.[1,2,3] There is growing evidence to indicate that these chemicals are produced during water chlorination[4,5], and consequently alternative strategies for water disinfection are being considered. Unfortunately, it is not known to what extent the mutagenic activity in chlorinated drinking water, and the associated potential health risks, are accounted for by chemicals identified thus far. Our laboratory has been exploring the use of humic acid for studying the mutagenic and carcinogenic properties of disinfection by-products.[6,7,8] The present report extends our findings on the mutagenic properties of chlorinated humic acids in the Ames test and includes results from studies on the ability of chlorinated and non-chlorinated humic acids to induce sister chromatid exchange (SCE) *in vitro* and to produce sperm-head abnormalities and micronuclei in bone marrow in mice *in vivo*.

Since disinfectant chemicals are generally added at levels sufficient to produce disinfectant residuals during distribution, the concern over potential health risks arising from the use of disinfectants may extend to the disinfectants themselves, or to by-products formed *in vivo*. This notion is supported by

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16. ABSTRACT

The identification of a number of mutagenic and carcinogenic chemicals in our public water supplies has raised concern over potential genetic and carcinogenic hazards to the human population. There is growing evidence to indicate that these chemicals are produced during water chlorination, and consequently alternative strategies for water disinfection are being considered. Unfortunately, it is not known to what extent the mutagenic activity in chlorinated drinking water, and the associated potential health risks, are accounted for by chemicals identified thus far. Our laboratory has been exploring the use of humic acid for studying the mutagenic and carcinogenic properties of disinfection by-products. The present report extends our findings on the mutagenic properties of chlorinated humic acids in the Ames test and includes results from studies on the ability of chlorinated and non-chlorinated humic acids to induce sister chromatid exchange (SCE) *in vitro* and to produce sperm-head abnormalities and micronuclei in bone marrow in mice *in vivo*. Since disinfectant chemicals are generally added at levels sufficient to produce disinfectant residuals during distribution, the concern over potential health risks arising from the use of disinfectants may extend to the disinfectants themselves, or to by-products formed *in vivo*. This notion is supported by results in bacterial assays which suggest that chlorine and monochloramine are capable of inducing DNA damage and causing mutation. In addition, halogenated organic compounds with known mutagenic and carcinogenic properties have been shown to be formed *in vivo* following oral dosing of rats with sodium hypochlorite. Because of these findings tests have been conducted on the mutagenic potential of various disinfectants *in vivo* by examining chromosomal damage in bone marrow and sperm-head abnormalities in mice. Studies are also being conducted on the toxicological properties of drinking water samples prepared using alternative techniques for disinfection and post-disinfection treatment. Preliminary results on the mutagenic activities of these samples are discussed.

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results in bacterial assays which suggest that chlorine and monochloramine are capable of inducing DNA damage and causing mutation.[9,10,11] In addition, halogenated organic compounds with known mutagenic and carcinogenic properties have been shown to be formed *in vivo* following oral dosing of rats with sodium hypochlorite.[12] Because of these findings tests have been conducted on the mutagenic potential of various disinfectants *in vivo* by examining chromosomal damage in bone marrow and sperm-head abnormalities in mice. The results of these tests are summarized here.

Studies are also being conducted on the toxicological properties of drinking water samples prepared using alternative techniques for disinfection and post-disinfection treatment. Preliminary results on the mutagenic activities of these samples are discussed.

MATERIALS

Humic acid solutions were prepared from Fluka humic acid (Fluka AG, Buchs, Switzerland) at a concentration of 1 g total organ carbon (TOC) per liter distilled water. Chlorination was performed at pH 7 or pH 11.5 without buffer, and at pH 7 with the addition of 0.25 M (final concentration) sodium phosphate buffer. At all pH reaction conditions a 1:1 molar ratio of chlorine (as Cl) to carbon (as TOC) was used. Further details of the methods for preparation and chlorination of the humic acids have been described previously.[7]

All other chemicals were purchased as reagent grade quality from commercial suppliers.

METHODS

In Vitro Bioassays

Mutagenicity assays were conducted with Salmonella typhimurium strains TA98 and TA100 according to the methods described by Maron and Ames[13]. Testing was performed at three or four dose levels using duplicate or triplicate plates per dose. Liver homogenate fraction (S9) was prepared from Aroclor 1254 treated, male Sprague-Dawley rats, and added to the S9 mix at a 5% (v/v) level. Assessment of a positive mutagenic response was based on a dose-related increase in the number of histidine revertant colonies which exceeded twice the background number of revertants for at least one dose level.

The methods used for evaluating the ability of test samples to induce SCEs in vitro using Chinese hamster ovary (CHO) cells have been described in detail elsewhere.[14] A one-tailed Student's t-test at the 0.01 level of significance was used to test for significant increases in the number of SCEs per cell over the concurrent control levels.

In Vivo Bioassays

The procedures for conducting the mouse bone marrow micronucleus and chromosomal aberration assays, and the mouse sperm morphology assay are described elsewhere.[15] Strain CD-1 mice

(Charles River Breeding Laboratories) were used for the micronucleus and chromosomal aberration assays, and B6C3F1 hybrid mice (Harlan Industries) were used for the sperm morphology assay. The same subchronic dosing regimen was used for studies involving both the disinfectant chemicals and the humic acid solutions. This involved administration of the dosing solutions by oral gavage at three dose levels (undiluted, 1:1 dilution, and 1:4 dilution), in five 1 ml doses on consecutive days.

Preparation of Water Samples Treated with Alternative Disinfectants

Mississippi River water which had been clarified and sand filtered was subjected to disinfection by treatment with either ozone, chlorine, chlorine dioxide, or monochloramine. The treatment contact time was 2 hours at initial disinfectant concentrations of 2-5 mg/l for chlorine, monochloramine, and chlorine dioxide, and 0.2-5 mg/l for ozone. Granular activated carbon (GAC) treatment of the chlorine treated water, with or without post-GAC rechlorination, were additional treatment options. Organic concentrates were prepared by passing the water samples (after acidification to pH 2) over XAD-8 and XAD-2 resins in sequence, eluting with acetone, and reducing the volume of the combined acetone eluates by rotary evaporation. Following removal of the acetone, the volume was adjusted with emulphor and distilled water to obtain a 4000-fold concentrate in 2% emulphor.

The samples were tested for mutagenicity in the Salmonella/ microsome mutagenicity assay.[13]

RESULTS AND DISCUSSION

Studies on Mutagenic Potential of Disinfectants

Chlorine, chlorine dioxide, monochloramine, chlorite, and chlorate were tested for their ability to produce chromosomal damage in a somatic cell line, as evaluated with the mouse bone marrow micronucleus and chromosmal aberration assays, and for their mutagenic potential to a germ cell line, as evaluated in the mouse sperm morphology assay. Since the species of chlorine which predominates during water treatment is pH dependent,[16] chlorine was prepared and tested at both pH 6.5 (where HOCl predominates) and at pH 8.5 (where the OCl^- predominates). Sodium chlorite and sodium chlorate were included in the testing of the disinfectants in order to determine whether any activity associated with chlorine dioxide might be due to the presence of chlorite or chlorate, which are major inorganic by-products formed upon addition of chlorine dioxide to water.[17] The results are summarized in Table I. The detailed data from these evaluations are discussed elsewhere.[18]

It can be seen that chlorine at pH 8.5 elicited a positive response in the mouse sperm morphology assay. No other effects with any of the disinfectants were noted. The effect with

chlorine, which was seen at the spermatocyte stage of spermatogenesis, was weak (about 2-fold above background at the maximum response), but reproducible, and statistically significant for at least two dose levels in both the initial and repeat trials. Failure to observe an effect with chlorine as OCl^- in the bone-marrow micronucleus or chromosomal aberration assays may reflect differences in assay sensitivities or differences in tissue distribution of OCl^- or its by-products. The work of Bruce and Heddle[18] seems to support the former explanation since results from their studies on 61 compounds indicated that the sperm assay has a greater success rate than the micronucleus assay for detecting agents that are active in vivo. Induction of sperm-head abnormalities in mice is not considered as definitive evidence of the mutagenicity or carcinogenicity of a chemical.[19] Nevertheless, the positive results obtained with chlorine raises concern over potential genetic and carcinogenic risks to the human population.

Because it seemed likely that a reaction product formed in vivo, rather than OCl^- itself, might be responsible for the effect on sperm morphology, we decided to examine for the presence of mutagenic activity in the urine from animals treated with chlorine at pH 8.5. A similar dosing regimen to that used in the sperm morphology assay was followed. In these experiments, rats were used in order to obtain sufficient volumes for adequate

testing. Salmonella/microsome mutagenicity assays were conducted on both unconcentrated urine and on 50-fold XAD-2 concentrates. Assays were performed both in the presence and absence of β-glucuronidase for detection of mutagens as their glucuronide conjugates. The results from these assays were negative, thus providing no evidence for the presence of mutagens in the urine.

Genotoxic Properties of Humic Acid Chlorination By-Products

In Vitro Studies

Previous work[6,7] had demonstrated that chlorination of humic acid resulted in the formation of direct-acting mutagens in the Ames test. The level of mutagenicity formed was dependent on the chlorination pH, although this observation appeared to be largely attributable to the instability of the mutagenic activity at alkaline pH. It was of interest to know if the genotoxic potential and the pH dependency of activity of chlorinated humic acid would be observed in other assay systems. Therefore, the same humic acid samples (before and after chlorination) were tested for mutagenic activity in the Ames test and SCE induction in CHO cells in vitro, and for micronuclei induction in mouse bone marrow and alteration of mouse sperm-head morphology in vivo.

Ames test results demonstrate the presence of mutagenic activity after, but not before, chlorination (Table II). It is also clear that the pH during chlorination has a substantial effect on the level of mutagenicity observed. The results from

testing these samples for SCE induction in CHO cells are also summarized in Table II. Each of the humic acid samples produced linear dose-dependent increases in the number of SCEs/cell. For all of the samples, at least the three highest responses were significantly greater than the concurrent control values at the 0.01 level of statistical significance. The slopes of the least-squares regression lines were used in determining the potencies of the samples for SCE induction. Comparison of the results in the Ames test and in the SCE assay reveals clear differences in the two assay systems for detection of activity of the samples. When expressed as a percentage of the activities of low pH (sample A), the levels of SCE induction at the higher pHs during chlorination (73% for sample B, 37% for sample C) are substantially higher than the corresponding mutagenic activities in the Ames test ((18-22% for sample A, 0-29% for sample B). Furthermore, there is clear evidence of SCE induction by non-chlorinated humic acids (sample D) whereas humic acids prior to chlorination show no evidence of mutagenicity. SCE induction is generally considered as evidence of primary DNA damage rather than mutation per se although there is generally a good correlation between the two endpoints with regard to the types of compounds detected.[20] The positive results showing SCE induction in a mammalian cell line provide confirmation of the genotoxic properties of chlorinated humic acids at pHs relevant to drinking

water chlorination pH, and demonstrate for the first time the genotoxic potential of non-chlorinated humic acid.

Figures 1-4 illustrate some of the problems that might occur when evaluating the mutagenic activity of complex mixtures using in vitro methods. In these experiments, known mutagens were added to the assay plates in the Ames test along with either chlorinated or non-chlorinated humic acids in order to see whether the expected additivity of response would be obtained. Figures 1 and 2 show that the mutagenic activities of 2-aminoanthracene (2-AA) and benzo(a)pyrene (B(a)P) were greatly suppressed by both chlorinated and non-chlorinated humic acids. Since both 2-AA and B(a)P require metabolic activation to become mutagenic, it occurred to us that the humic materials might interfere with one or more components of the S9 activation mixture. However, inhibition of the activities of two direct-acting mutagens, sodium azide and 2-nitrofluorene, was also observed (Figures 3 and 4). In order to better compare the extent of inhibition among the four known mutagens, the observed vs. expected activities were determined and the percent inhibition calculated. The data in Table III indicate the extent of inhibition is compound dependent and also is dependent on the nature of the humic material (chlorinated vs. non-chlorinated). Overall it appears that the activities of the two promutagens (those requiring S9 activation) were inhibited to a greater degree than those of the two direct-acting compounds.

These results suggest that components both in chlorinated and unfinished water might interfere with the metabolic activation capacity of the S9 mixture, and this interference may mask the presence of promutagens. Humic acids are known to chelate metal ions,[21] including Mg^{++}, which is a cofactor in the S9 mix needed for NADPH generation. Humic materials are also known to bind other organics[21] and such binding could serve to make direct-acting mutagens or mutagenic metabolities inaccessible to DNA binding. In the case of the chlorinated humic acid samples, which are toxic (i.e., lethal) as well as mutagenic, the toxicity may also contribute to the inhibition of activity. Regardless of the reasons for the inhibition, the present experiments clearly demonstrate antagonism by chlorinated and non-chlorinated humic acids towards the in vitro mutagenic activity of known mutagens. In view of these results, the potential for antagonism among consitutents of raw and finished water seems a distinct possibility. Since such antagonism could have a substantial impact on mutagenicity assay results and their interpretation, we believe this finding warrants further attention in mutagenicity studies of water samples.

Previous results from testing humic materials obtained from two natural sources as well as a commercial humic source indicated that the mutagenic activity formed following chlorination was decreased to varying extents by the addition of S9 mix to the

assay.[6,7] This observation is also common in mutagenicity studies done on drinking water concentrates.[3] Eder et al.[22] have suggested that the glutathione (GSH) in liver S9 preparations might be responsible for the reduction of mutagenicity of direct-acting allylic compounds by S9. Since chlorallylic compounds have been identified in chlorinated humic acid solutions,[23] we decided to see whether GSH might be responsible for the S9 inactivation of mutagens in chlorinated humic acids. The results in Figure 5 show that a substantial portion of the mutagenic activity is quenched by GSH. The biphasic shape of the inhibition curve without S9 indicates the presence of mutagenic compounds that are highly sensitive to GSH, as well as relatively insensitive compounds. The fact that much less reduction in activity by GSH occurs when S9 is present is probably attributable to the fact that the activity of GSH sensitive compounds have already been quenched by the S9. Based on levels of 1.3-1.7 mM GSH in S9 mix (i.e. approximately 230 µg/plate) which were reported by Eder et al.,[22] it appeared that the degree of inactivation seen with S9 alone (Figure 5) could be largely accounted for by the GSH present. In order to confirm this possibility, GSH concentrations were measured in our S9 using the method of Hissin and Hilf[24] and were found to be 1.27 mM in the S9 fraction, or about 5 mM in the whole liver. This value agrees well with reported values for rat liver which are typically 2-10 mM.[25] Although the levels of S9

fraction in S9 mix were not indicated by Eder et al.,[22] the levels generally used are 4-10% (v/v). This would mean that the GSH levels in S9 mix reported by Eder et al.[22] are 10-25 times higher than ours. The reason for this discrepancy is unknown but may reflect an error in terminology by Eder et al.[22] (i.e. S9 mix vs. S9 fraction). In any case, the level of GSH in our assays with S9 amounts to only about 10 µg per plate, which would account for only a slight reduction in mutagenicity of chlorinated humic acid (Figure 5). Thus, GSH appears to be of only minor importance in the mutagen inactivation by S9.

In Vivo Studies

The results from testing the chlorinated and non-chlorinated humic acid samples in the mouse micronucleus assay are shown in Table IV. There were no significant increases in the percent of micronucleated cells above control values for any of the samples tested, thus providing no evidence for clastogenic effects on mouse bone marrow in vivo. Similarly, the results from testing the samples in the mouse sperm morphology assay were also negative (Table V) thus providing no evidence for the mutagenic potential of these samples in vivo.

The reason for the discrepancies between the in vivo and in vitro assay results is not known but several explanations are possible. The recommendations made by the respective Gene-Tox groups for the micronucleus assay[26] and the sperm morphology

assay[19] are that testing be conducted with doses approaching LD_{50} in order for the test to be considered adequate. The dose levels administered in the present studies were limited by the solubility of humic acid and the maximum volumes which could be administered. The highest dose levels for any of the samples produced no overt signs of toxicity or lethality. Thus, the negative results obtained under the conditions of our assays should probably be regarded as inconclusive. The same logic would apply to the disinfectant studies in vivo where no lethality of dosed animals was observed. The other explanation for the negative results in vivo might be that the direct-acting mutagens may be effectively detoxified during in vivo metabolism. As already indicated, liver S9 preparations have been found to decrease the mutagenicity of chlorinated humic acids in vitro. It seems reasonable to expect that similar inactivation might also occur in vivo. Although GSH does not appear to be important for inactivtion of mutagens by S9 in vitro, it should be noted that the observations of significant GSH inactivation at levels above 100 µg/plate (0.1 mM in the top agar) in vitro may have substantial importance for detoxification in vivo where liver and kidney GSH levels are 20 to 100 times higher.[25] Thus, failure to observe any effects in the two in vivo assays may be accounted for both by the in vivo detoxification of the mutagens, and the failure to administer high enough doses to produce an effect. In

addition, the two assays employed will only detect effects on bone marrow and testes. There may be specificity in the tissue distribution of the active compounds which may make these tissues unlikely targets.

Studies on Alternative Disinfectant By-Products

For assessing the relative safety of chlorine vs. other chemicals used for disinfection, studies are being conducted to examine the mutagenic activity of water before and after treatment with various dsinfectants. Preliminary results from these studies are presented in Table VI. Based on a 2-fold above background criterion for assessment of a positive response (i.e., induced/control revertants > 1.0), it is clear that both chlorine and monochloramine treated water exhibit reproducible mutagenicities for strains TA98 and TA100. The dose-response curves in both cases were essentially linear over the dose ranges tested. Treatment with GAC which had been in use for about 7 months removed the mutagenic activity of chlorine treated water; however, rechlorination after GAC resulted in the reformation of about one-third of the activity. This latter result is in contrast to the findings of Loper et al.[27] who found that both the mutagenicity and mutagen forming potential of chlorinated Ohio River water were completely removed by GAC which had been in use for a similar period of time.

To our knowledge the mutagenicity of chloramine treated water has not been previously reported. This finding should be taken into consideration in the overall safety assessment of disinfectant treatment alternatives.

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This article has been reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Mention of trade names or commercial products does not constitute endorsements or recommendation for use.

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Table I. Evaluation of Mutagenic and/or Carcinogenic Potential of Disinfectants in Three *In Vivo* Bioassays

Disinfectant	Mouse Micronucleus Assay	Mouse Bone Marrow Cytogenetics Assay	Mouse Sperm-Head Abnormality Assay
Chlorine, pH 6.5 (HOCl)	-	-	-
Chlorine, pH 8.5 (OCl^-)	-	-	+[a]
Monochloramine	-	-	-
Chlorine Dioxide	-	-	-
Chlorite	-	-	-
Chlorate	-	-	-

[a]Based on reproducible, dose-related, and statistically significant elevations in % sperm-head abnormalities over concurrent control values at the three week post-treatment kill time.

Table II. pH Dependence of Mutagenic Activity in the Ames Test and SCE Induction in CHO Cells Upon Treatment of Humic Acid with Chlorine (HOCl/OCl)

Humic Acid Sample	Mutagenic Activity[a]		SCE Induction[b]
	TA98	TA100	
A - Chlorinated (pH: 7.0 → 2.5)	339 ± 29 (100)	1696 ± 148 (100)	2.96 (100)
B - Chlorinated[c] (pH: 7.0 → 6.5)	62 ± 10 (18)	367 ± 34 (22)	2.15 (73)
C - Chlorinated (pH: 11.5 → 6.5)	N.S.[d]	490 ± 33 (29)	1.10 (37)
D - Non-Chlorinated[e]	N.S.[d]	N.S.[d]	0.62 (21)

[a]Net revertants/ml of sample, calculated from the linear portion of the dose response curve, from assays without S9 added. ( ) indicates the percent of activity in sample A.

[b]SCEs/cell/% sample incorporated into medium, calculated from the linear portion of the dose response curve, from assays without S9 added. ( ) indicates the percent of activity in sample A.

[c]0.25 M NaPi buffer was added during chlorination to stabilize the pH.

[d]N.S. = Not significant (i.e., less than 2-fold above background at any dose tested).

[e]The non-chlorinated humic acid was prepared at pH 7 and then pH was lowered to pH 2.5 with HCl.

Table III. Effect of Chlorinated and Non-Chlorinated Humic Acids on Activity of Known Mutagens in the Ames Test

Humic Acid Sample	Amount Sample Added (µls/ Plate)	% Inhibition of Mutagenicity					
		TA98			TA100		
		2-NF	2-Aa	B(a)P	NaN_3	2-AA	B(a)P
Non-Chlorinated	100	10	7	72	18	-19	23
	200	37	40	99	18	49	69
	400	41	79	100	10	98	100
Chlorinated	100	34	28	39	37	28	-17
	200	59	57	55	44	60	11
	400	59	98	65	42	81	55

2-NF = 2-nitrofluorene; 2-AA = 2 aminoanthracene; B(a)P = benzo(a) pyrene; NaN_3 = sodium azide.

Table IV. Activity of Chlorinated and Non-Chlorinated Humic Acids in the Mouse Micronucleus Assay

Humic Acid Sample	% Micronucleated Cells[a]			
	Dose [b]			
	0	1:4	1:1	Undiluted
Non-Chlorinated	0.25 ± 0.06	0.23 ± 0.03	0.40 ± 0.09	0.24 ± 0.06
Chlorinated (pH 7.0 + 2.5)	0.43 ± 0.12	0.24 ± 0.06	0.39 ± 0.12	0.24 ± 0.09
Chlorinated (pH 7.0 + 6.5)	0.47 ± 0.08	0.41 ± 0.17	0.40 ± 0.14	0.33 ± 0.08
Chlorinated (pH 11.5 + 6.5)	0.74 ± 0.09	0.41 ± 0.05	0.38 ± 0.07	0.33 ± 0.07

[a]Mean per animal ± standard error of the mean based on 10 animals per dose group (5 males + 5 females).
[b]Animals received 5 consecutive daily administrations of the humic acid solutions at the indicated concentrations in 1 ml volumes by oral gavage.

Table V. Activity of Chlorinated and Non-Chlorinated Humic Acids in the Mouse Sperm-Head Abnormality Assay

Humic Acid Sample	Sacrifice Time (Wks)	% Sperm-Head Abnormalities[a] Dose[b] 0	1:4	1:1	Undiluted
Non-Chlorinated	3	1.49 ± 0.14	1.05 ± 0.12	0.83 ± 0.15	1.00 ± 0.11
	5	1.03 ± 0.11	0.75 ± 0.07	0.82 ± 0.12	0.90 ± 0.10
Chlorinated (pH 7.0 → 2.5)	3	0.75 ± 0.11	0.87 ± 0.10	0.92 ± 0.11	0.99 ± 0.13
	5	0.89 ± 0.09	0.86 ± 0.08	0.76 ± 0.10	1.27 ± 0.20
Chlorinated[c] (pH 7.0 → 6.5)	3	0.72 ± 0.10	0.74 ± 0.13	1.12 ± 0.14	1.02 ± 0.12
	5	0.96 ± 0.09	1.12 ± 0.12	0.88 ± 0.07	0.96 ± 0.09
Chlorinated (pH 11.5 → 6.5)	3	0.81 ± 0.12	0.75 ± 0.10	0.88 ± 0.15	1.00 ± 0.11
	5	0.71 ± 0.10	0.84 ± 0.07	0.69 ± 0.05	1.20 ± 0.12

[a]Mean per animal ± standard error of the mean based on 10 animals per dose group.
[b]The dose schedule was the same as that used for the mouse micronucleus assay.

Table VI. Mutagenic Activity of Organic Concentrates from Raw and Treated Mississippi River Water in *Salmonella typhimurium* Strains TA98 and TA100 in the Absence of Metabolic Activation (S9)

	Induced Revertants/Control Revertants[a]			
	TA98		TA100	
Sample	Trial 1	Trial 2	Trial 1	Trial 2
Influent	0.55	0.08	0.20	-0.06
Ozone	0.15	-0.18	-0.07	-0.33
Chlorine Dioxide	1.10	0.29	0.37	0.12
Chloramine	2.10	0.69	1.82	1.09
Chlorine	3.00	2.33	3.26	2.77
Chlorine & GAC	0.20	0.12	0.24	0.03
Chlorine & GAC & Rechlorination	1.15	0.49	1.45	1.21

[a]Average of the net revertants/control revertants observed in duplicate plates treated with 100 µl of a 4000 x concentrate produced by combined XAD-2 and XAD-8 resins. Trials indicate independent experiments.

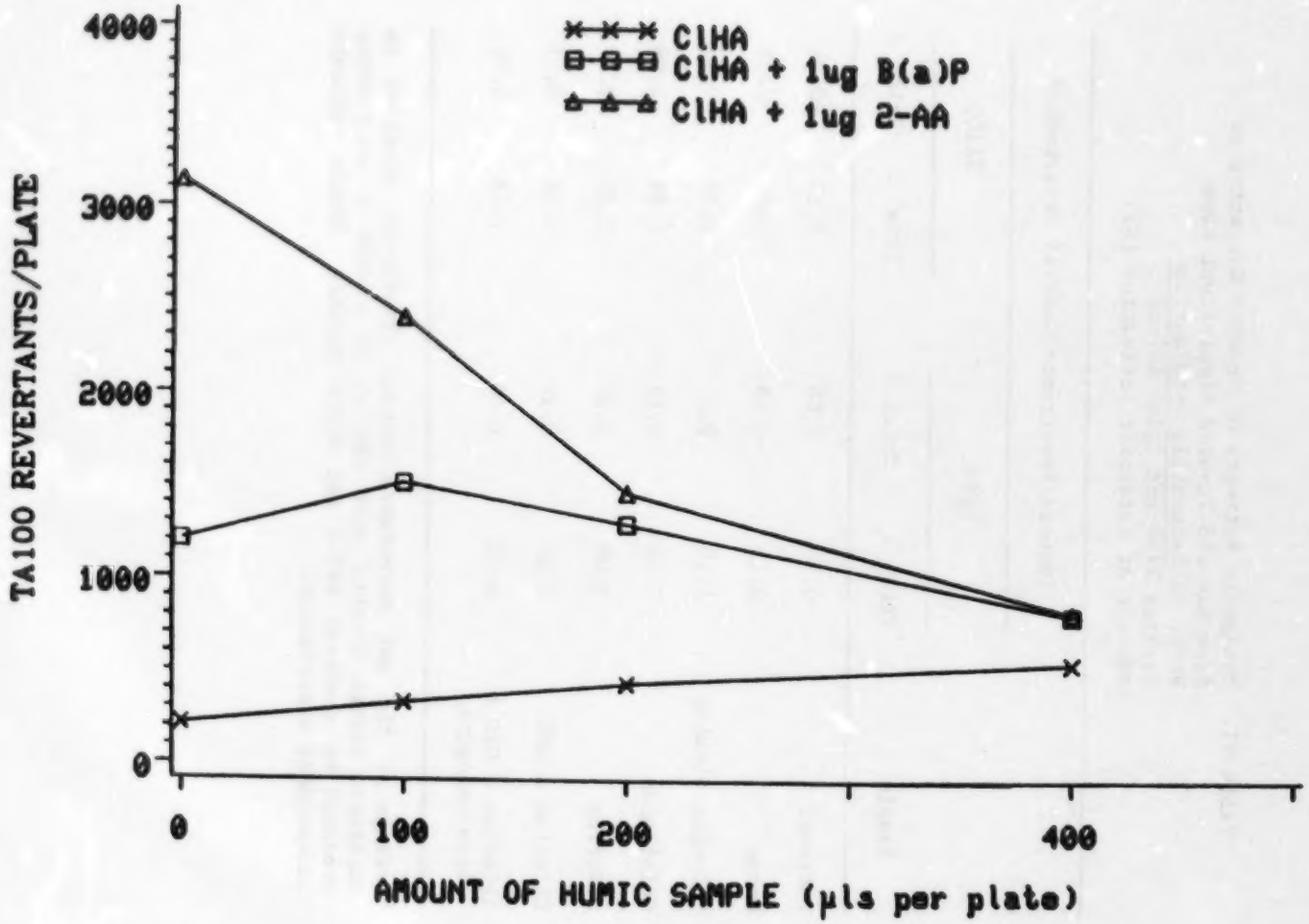

Figure 1. Inhibition of the mutagenicity of 2-aminoanthracene and benzo(a)pyrene for strain TA100 by chlorinated humic acid.

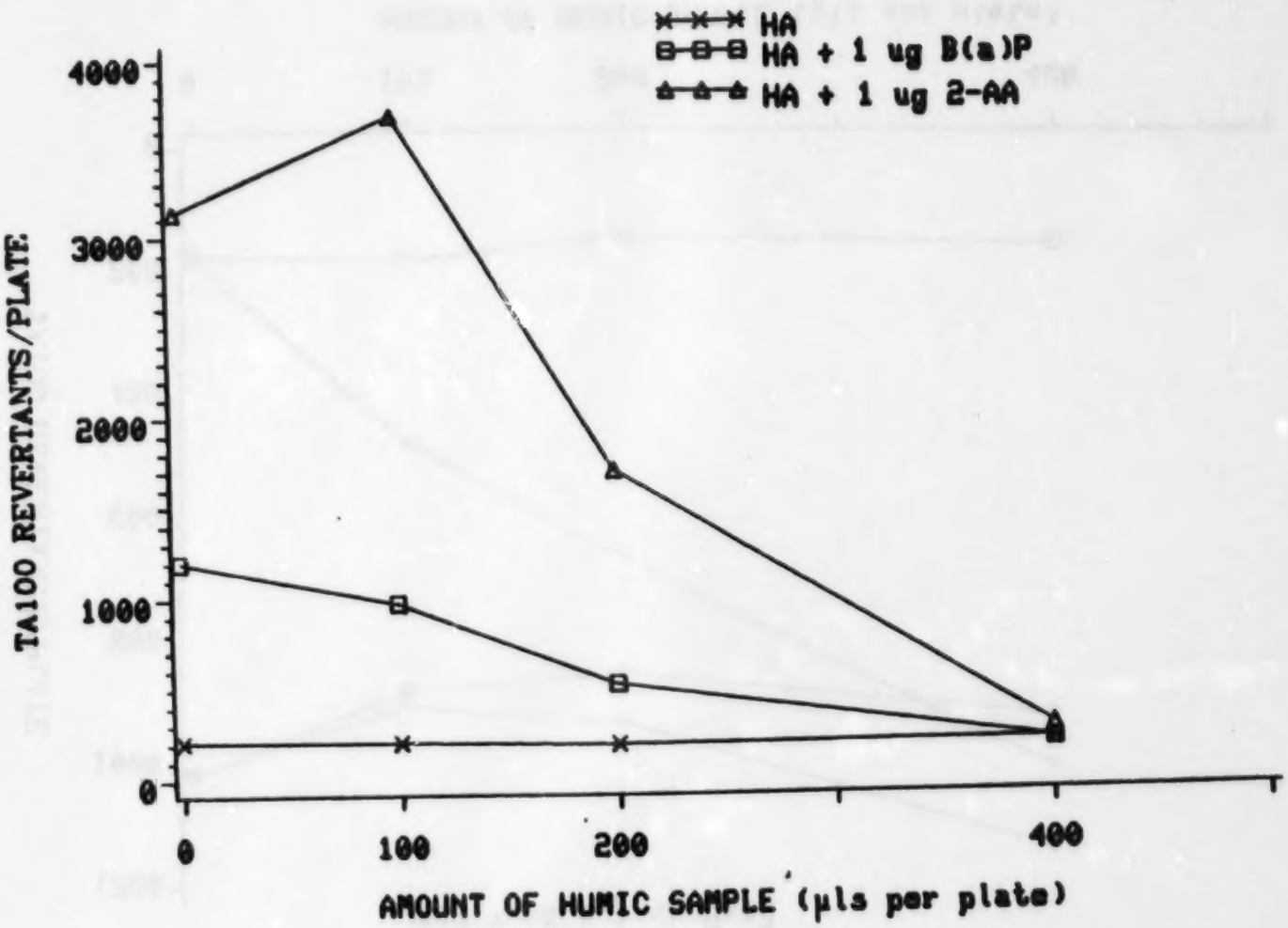

Figure 2. Inhibition of the mutagenicity of 2-aminoanthracene and benzo(a)pyrene for strain TA100 by non-chlorinated humic acid.

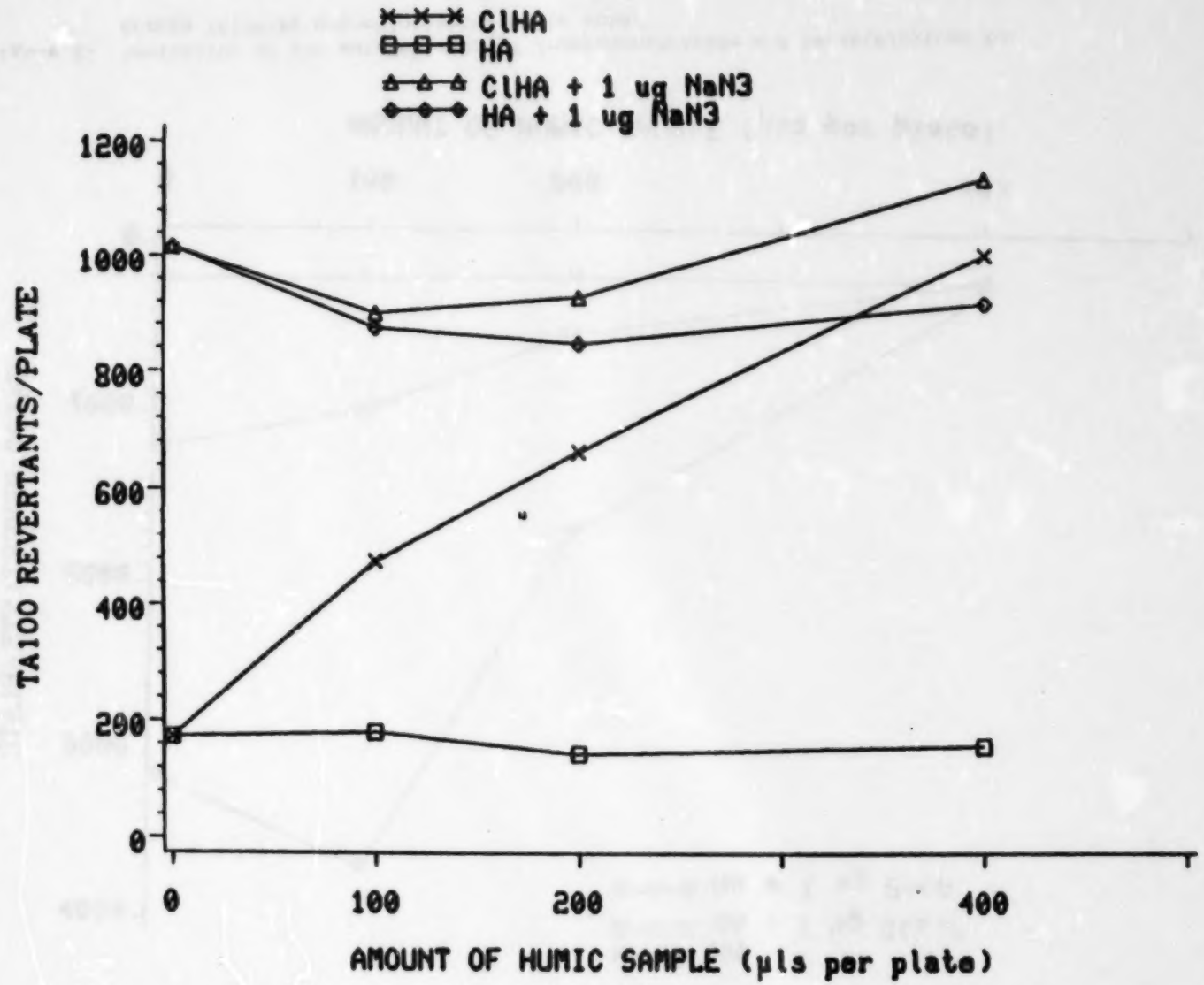

Figure 3. Effect of chlorinated and non-chlorinated humic acids on the direct-acting mutagenicity of sodium azide for strain TA100.

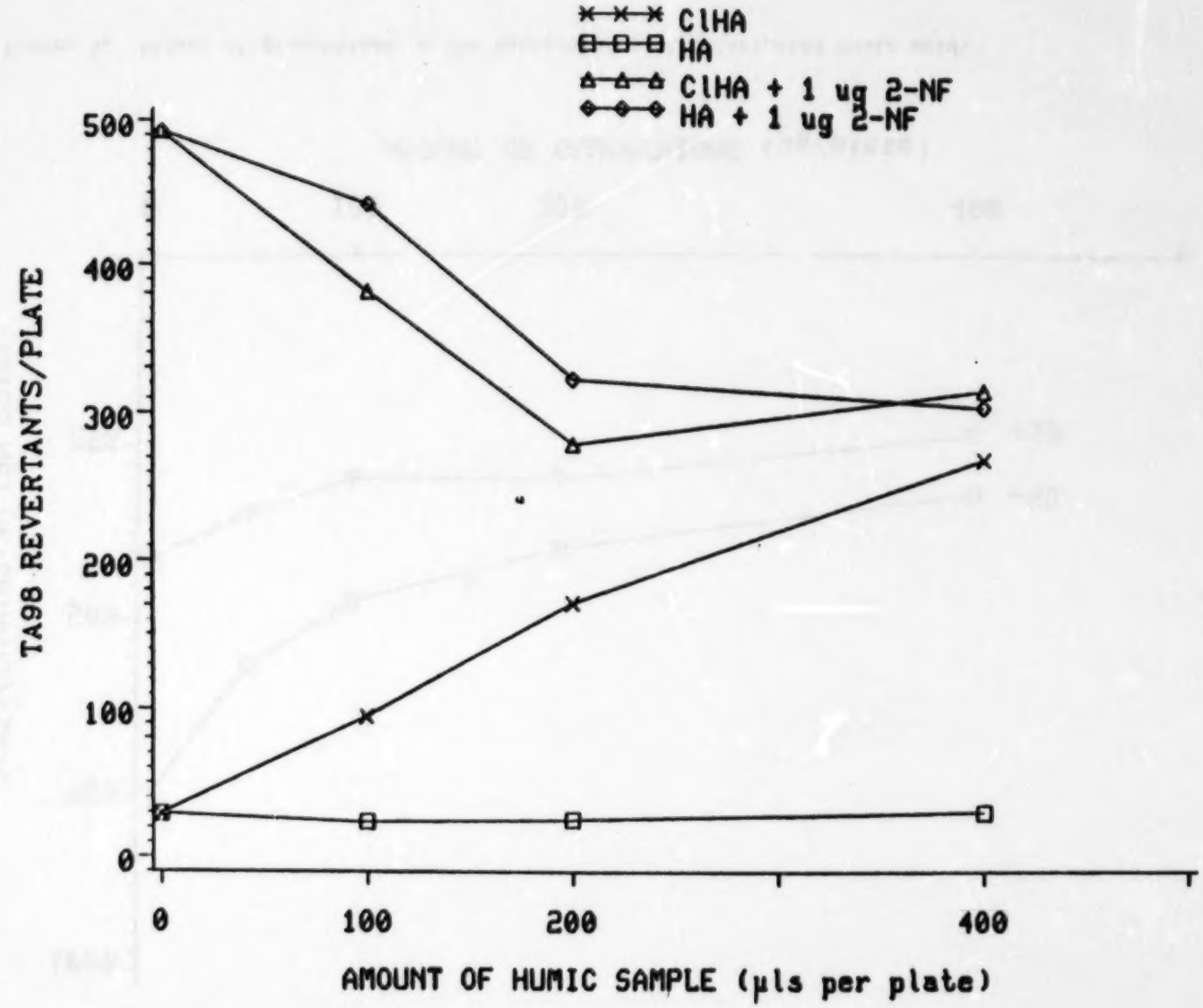

Figure 4. Effect of chlorinated and non-chlorinated humic acids on the direct-acting mutagenicity of 2-nitrofluorene for strain TA98.

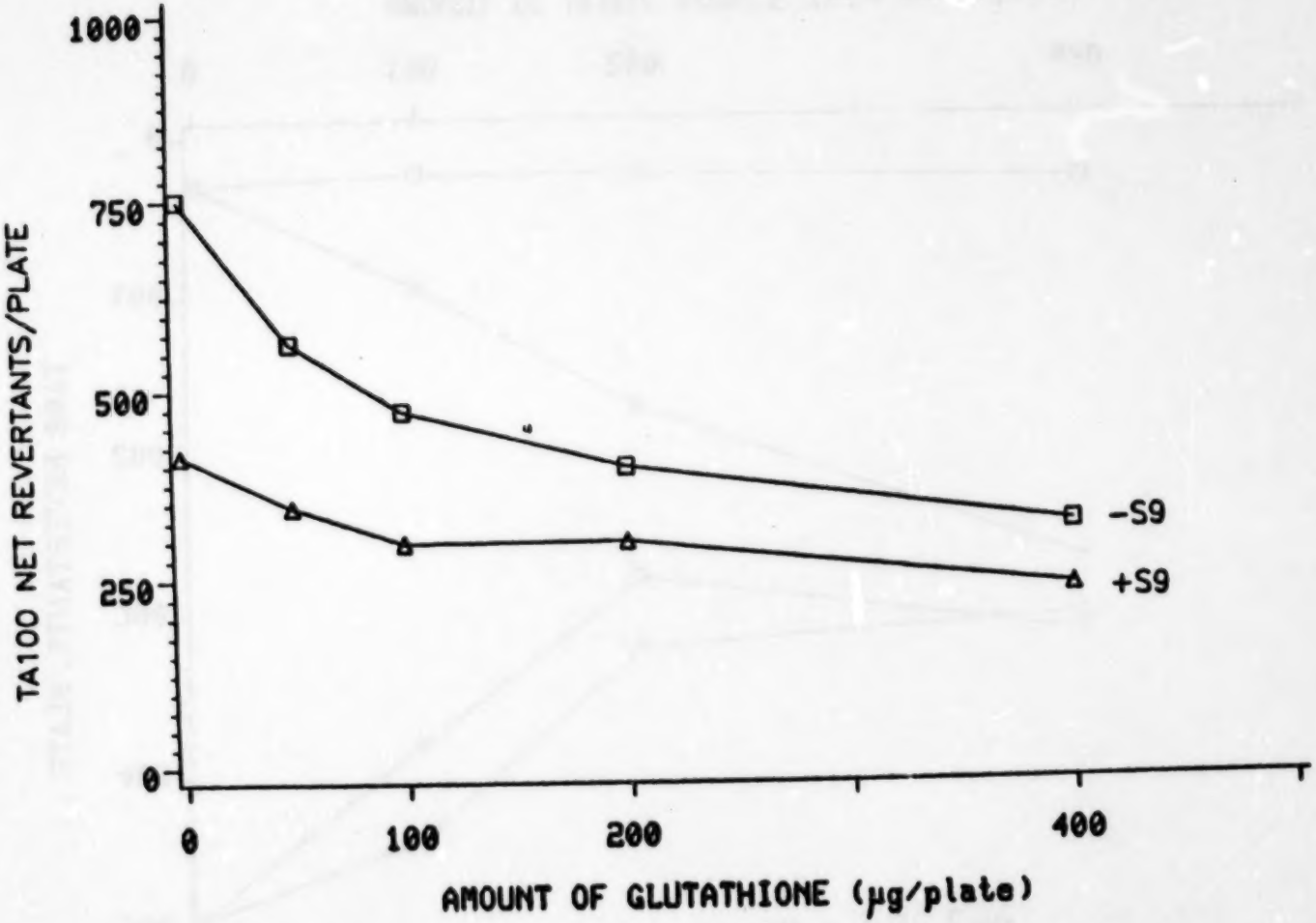

Figure 5. Effect of glutathione on the mutagenicity of chlorinated humic acid.